Claims

1. (currently amended) A test strip for determining presence and/or amount of an analyte in a liquid sample comprising:

a sample application area

a mobilization zone;

a mobile or mobilizable detectable tracer in the mobilization zone;

a primary capture area comprising a first immobilized binding partner having a binding affinity for the analyte and a binding affinity for the detectable tracer; and

a secondary capture area comprising a second immobilized binding partner having a binding affinity for the detectable tracer,

wherein the sample application area, mobilization zone, primary capture area and secondary capture area are in a path of liquid flow ~~along~~ through a bibulous substrate from the sample application area distally through the mobilization zone to the primary capture area and then to the secondary capture area, wherein the ~~bibulous substrate~~ mobilization zone comprises a porous material to which the detectable tracer has been applied in the presence of a delayed release agent ~~selectively delays migration of the detectable tracer~~ so that the analyte migrates through the porous material of the bibulous substrate ahead of the detectable tracer and a distal flow of analyte reaches the primary capture area before a distal flow of detectable tracer reaches the primary capture area such that subsequent binding of detectable tracer to first immobilized binding partner is inhibited and unbound detectable tracer continues along the path of flow distally to the second immobilized binding partner to provide a signal from the secondary capture area that indicates the presence of the analyte in the liquid sample.

2. (previously presented) The test strip of claim 1, wherein the detectable tracer is present on the test strip in a position that selectively delays migration of the detectable tracer through the bibulous substrate so that the detectable tracer reaches the primary capture area after the analyte reaches the primary capture area.

3. (canceled)

4. (currently amended) The test strip of claim 3, wherein the test strip is a lateral flow chromatography strip comprising a bibulous collection member with a proximal edge and a distal edge ~~mobilization zone is a bibulous substrate~~.

5. (currently amended) The test strip of claim 4, wherein the ~~test strip is a~~ porous ~~strip~~ material comprises pores, and the detectable tracer is larger than the analyte but ~~sufficiently~~ smaller than pores of the porous strip to allow the detectable tracer to migrate along the path of liquid flow through the bibulous porous strip more slowly than the analyte migrates along the path of liquid flow.

6. (currently amended) The test strip of claim 4, wherein the test strip comprises a bibulous liquid collection member that contains the mobilization zone, primary capture area and secondary capture area, and the detectable tracer comprises an analyte analog ~~positioned in~~ that has been applied as a liquid to the mobilization zone of the collection member where it has dried beneath the surface of the test strip along which the liquid sample migrates from the mobilization zone to the primary and secondary capture area such that the detectable tracer migrates through the test strip to the primary capture area more slowly than analyte in the liquid sample that migrates along the surface of the test strip.

7. (previously presented) The test strip of claim 4, wherein the tracer has a polarity or charge that interacts with the bibulous substrate to retard migration of the tracer relative to migration of the analyte.

8. (previously presented) The test strip of claim 3, wherein the test strip contains in the mobilization zone at least one reagent that selectively delays release of the tracer along the path of liquid flow relative to migration of the analyte along the path of liquid flow.

9. (previously presented) The test strip of claim 8, wherein the at least one reagent is selected from the group consisting of sucrose, mannitol, glycerol, polyvinyl alcohol (PVA), polyvinyl pyrrolidone (PVP), and mixtures thereof.

10. (previously presented) The test strip of claim ~~1~~ 9, wherein the reagent comprises 5-50% sucrose, 5-30% mannitol, 1-15% glycerol, 0.1-5% polyvinyl pyrrolidone, or mixtures thereof.

11. (previously presented) The test strip of claim 1, wherein the mobilization zone is below the sample application zone.

12. (previously presented) The test strip of claim 1, wherein the first and second immobilized binding partners are each antibodies, and the detectable tracer comprises an analyte analog, and the first binding partner is an antibody having a greater affinity for the analyte than the analyte analog.

13. (previously presented) The test strip of claim 1, wherein the detectable tracer comprises an analyte analog.

14. (previously presented) The test strip of claim 1, wherein the detectable tracer comprises a visually detectable label covalently attached to analyte or an analyte analog.

15. (previously presented) The test strip of claim 1, wherein the detectable tracer comprises a detectable tracer for an analyte selected from the group consisting of an antigen of an infectious disease, an antigen to an antibody of an infections disease, a hormone, a growth factor, a therapeutic drug, a drug of abuse, a product of the metabolism of a drug of abuse, and a hapten.

16. (previously presented) The test strip of claim 15, wherein the detectable tracer comprises a detectable tracer for an analyte comprising an antibody selected from the group consisting of an antibody to Human Immunodeficiency Virus (HIV), an antibody to Human T-Cell Lymphotropic Virus (HTLV), an antibody to *Helicobacter pylori*, an antibody to hepatitis, an antibody to measles, an antibody to mumps, and an antibody to rubella.

17. (previously presented) The test strip of claim 15, wherein the detectable tracer comprises a detectable tracer for an analyte comprising a therapeutic drug or drug of abuse or products of the metabolism of a drug of abuse, wherein the analyte is selected from the group consisting of tetrahydrocannabinol, nicotine, cotinine, ethanol, theophylline, phenytoin, acetaminophen, lithium, diazepam, nortryptyline, secobarbital, and phenobarbitol, methamphetamine and fragments, mimetics, and analogs or derivatives thereof.

18. (previously presented) The test strip of claim 17, wherein the detectable tracer comprises a detectable tracer conjugate for an analyte that is a product of metabolism of a drug of abuse, and the product of metabolism comprises cotinine.

19. (previously presented) The test strip of claim 15, wherein the detectable tracer comprises a detectable tracer conjugate for an analyte comprising a hormone, and the hormone is selected from the group consisting of testosterone, estradiol, estriol, 17-hydroxyprogesterone, progesterone, thyroxine, thyroid stimulating hormone, follicle stimulating hormone, and luteinizing hormone, and fragments, mimetics, analogs or derivatives thereof.

20. (previously presented) The test strip of claim 1, wherein the quantity of detectable tracer binding to the secondary capture area, and by correlation the amount of the analyte in the liquid sample, is indicated by intensity of a detection signal of the detectable tracer in the secondary capture area.

21. (previously presented) The test strip of claim 1, wherein the secondary binding area of the test strip is divided into at least two discrete and non-overlapping bands, wherein the quantity of tracer binding to the secondary capture area, and by correlation the amount of the analyte in a tested sample, is indicated by the number of bands to which the tracer molecule binds.

Claims 22-30 (canceled)

31. (previously presented) A method for detecting and/or quantitating an analyte in a liquid sample, comprising:

contacting the liquid sample with the sample application area of the test strip of claim 1, and allowing the liquid sample to mobilize the tracer such that the distal flow of tracer migrates with the liquid sample, but reaches the primary capture area after distal flow of analyte in the liquid sample;

wherein the distal flow of analyte that reaches the primary capture area occupies first immobilized binding partner such that subsequent binding of the detectable tracer to the first immobilized binding partner is inhibited, whereby unbound detectable tracer continues along the path of flow distally to bind to the second immobilized binding partner and provide a signal from the secondary capture area that indicates the presence of the analyte in the liquid sample; and

detecting a signal from the secondary capture area to detect and/or quantitate the analyte.

Claims 32-34 (canceled)

35. (previously presented) The method of claim 31, further comprising quantifying an amount of analyte in the liquid sample, wherein the amount of analyte in the liquid sample determines an intensity of the signal from the tracer in the secondary capture area.

36. (previously presented) The method of claim 31, wherein the liquid sample migrates from the mobilization zone along the test strip in the path of liquid flow by capillary action and the mobilization zone is bibulous.

37. (original) The method of claim 31, wherein the analyte has a molecular weight of about 100 – 1,000 Daltons.

38. (original) The method of claim 31, wherein the analyte has a molecular weight of greater than 1,000 Daltons.

39. (canceled)

40. (previously presented) The method of claim 31, wherein the liquid sample is selected from the group consisting of urine, blood, tears, sweat and saliva.

41. (previously presented) The method of claim 40, wherein the liquid sample is saliva.

42. (previously presented) The method of claim 41, wherein the saliva is combined with a bile acid or bile salt in a concentration that reduces occurrence of false positives in the immunoassay.

43. (previously presented) The method of claim 42, wherein the bile acid or bile salt ranges in concentration from about 0.1 weight percent to about 1.0 weight percent of the saliva/bile salt or saliva/bile acid combination.

44. (previously presented) The method of claim 43, further comprising contacting a chelator of divalent cations with the saliva sample.

45. (previously presented) A test kit for the detection and/or the determination of an analyte in a sample comprising:

(a) the test strip of claim 1; and

(b) instructions for using the test strip such that the flow of analyte in the liquid sample reaches the primary capture zone before the flow of tracer.

46. (canceled)

47. (currently amended) The method of claim 31, wherein the detectable tracer is ~~contained~~ dried beneath an external surface of the test strip and the sample application area is a pad that is applied over the detectable tracer dried on the strip, and the liquid sample is applied to the ~~external surface of the test strip~~ pad, such that the flow of detectable tracer migrates from the pad through the mobilization zone to the primary capture area at a slower rate along the path of flow than flow of analyte in the liquid sample migrates along the path of flow toward the primary capture area.

48. (canceled)

49. (previously presented) The method of claim 31, wherein the mobilization zone is in a bibulous substrate wherein the detectable tracer interacts with the test strip to slow flow of the detectable tracer along the path of liquid flow more than flow of analyte in the liquid sample is slowed such that any analyte in the liquid sample reaches the primary capture zone ahead of the detectable tracer.

Claims 50-74 (canceled)

75. (currently amended) A test strip for determining presence and or amount of an analyte in a liquid sample, comprising:

a bibulous substrate comprising a sample application zone, a mobilization zone that comprises a delayed release mobilizable detectable tracer, a primary capture zone that comprises a first immobilized binding partner having a binding affinity for the analyte and a binding affinity for the detectable tracer, and a secondary capture area that comprises a second immobilized binding partner having a binding affinity for the detectable tracer, wherein the mobilizable detectable tracer comprises an analyte or analyte analog;

wherein the sample application area, mobilization area, primary capture area and secondary capture area are in a path of liquid flow ~~along~~ through the bibulous substrate from the sample application area distally through the mobilization zone to the primary capture area and then to the secondary capture area, wherein release of the detectable tracer from the bibulous substrate is delayed relative to movement of analyte by a delayed release agent in the

mobilization zone as liquid flows along the path of liquid flow so that analyte migrates in advance of the detectable tracer and a distal flow of analyte reaches the primary capture area before a distal flow of detectable tracer reaches the primary capture area such that subsequent binding of detectable tracer to first immobilized binding partner is inhibited and unbound detectable tracer continues along the path of flow distally to the second immobilized binding partner to provide a signal from the secondary capture area that indicates the presence of the analyte in the liquid sample.

76. (new) The test strip of claim 75, wherein the detectable tracer comprises a dried liquid that has been applied to the mobilization zone of the bibulous substrate.

77. (new) The test strip of claim 75, wherein the sample application zone is a pad that completely overlaps the mobilization zone.

78. (new) The test strip of claim 75, wherein the sample application zone is a pad that partially overlaps the mobilization zone.

79. (new) The test strip of claim 76, wherein the detectable tracer comprises multiple dried lines of liquid, and the sample application zone is a pad that overlaps only the first dried line of liquid.

80. (new) The test strip of claim 76, wherein the detectable tracer in the mobilization zone is distal to the sample application zone and not beneath the sample application zone.

81. (new) The test strip of claim 76, wherein the delayed release reagent is selected from the group consisting of sucrose, mannitol, glycerol, polyvinyl alcohol (PVA), polyvinyl pyrrolidone (PVP), and mixtures thereof.

82. (new) The test strip of claim 81, wherein the reagent comprises 5-50% sucrose, 5-30% mannitol, 1-15% glycerol, 0.1-5% polyvinyl pyrrolidone, or mixtures thereof.

83. (new) The test strip of claim 82, wherein the reagent comprises 5-50% sucrose.